REMARKS

In accordance with the present invention, there are provided methods for analyzing complex protein mixtures. Invention methods are useful for a variety of purposes including provision of diagnostic information concerning pathogenic states, identification of proteins that may act as therapeutic targets, drug discovery, and the like.

By the present communication, claims 1, 8, 11 and 37 have been amended to define Applicant's invention with greater particularity. Specifically, claims 8 and 11 have been amended to reflect grammatical changes suggested by the Examiner. The Examiner's identification of these inadvertent informalities is acknowledged with appreciation. No new matter has been introduced by the subject amendments as the amended claim language is fully supported by the specification and original claims.

In view of the amendments submitted herewith, claims 1-6 and 8-37 remain pending in this application, with claims 1-6, 8-13 and 37 under active prosecution. A detailed listing of all claims that are, or were, in the application is presented herewith, beginning on page 2, along with an appropriate status identifier.

The rejection of claims 1-6, 8-13, and 37 under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement is respectfully traversed. Specifically, Applicant respectfully disagrees with the Examiner's assertion (page 2, item 5, lines 5-6) that:

> There is no written description in the specification for the claim 1 steps of "isolating ...by binding to a receptor bound to a solid phase... removing unbound proteins, and releasing".

The present invention provides methods for analyzing a complex protein mixture, said methods comprising multiple steps, including the steps of:

labeling one or more active target proteins present in said complex protein mixture by combining at least one probe, said probe(s) comprising a functional group specific for one or more active target proteins and a fluorescent moiety, with said complex protein mixture under conditions whereby said probe(s) covalently react with said active target proteins,

isolating one or more of said labeled active target proteins by binding to a receptor bound to a solid phase, wherein said receptor binds the probe labeling said active target proteins,

removing unbound proteins,

releasing bound labeled active target proteins from said receptor, and

detecting a signal from one or more labeled active target proteins present in said complex protein mixture following said isolating, wherein said signal is detected by separating one or more of said labeled active target proteins and generating a fluorescent signal from or more of said labeled active target proteins during or following said separation.

Contrary to the Examiner's assertion, the individual steps contemplated for use in the practice of the present invention, e.g., binding of a target protein within a protein mixture to immobilized receptor, washing away non-binding proteins, and releasing of target protein, are well known in the art. As witness to the maturity of this procedure, Applicant employed Affi-gel Hz (Bio-Rad) beads coupled to monoclonal anti-TMR antibodies which were coupled according to manufacturer's recommendations (Specification, paragraph 179). The capture steps recited in claim 1 are exemplified in the Specification (paragraph 171-185). These procedures would be readily understood by those of skill in the art, and could be understood and reproduced by those of skill in the art without undue experimentation. Furthermore, the Specification teaches (Example 7, paragraphs 187-192) the binding to a solid-phase bound receptor, removal of unbound protein, and release of bound ligand, thereby provided additional written description.

Thus, contrary to the Examiner's assertion, the specification fully meets the requirements of 35 USC § 112, first paragraph.

The rejection of claims 1-6, 8-13, and 37 under 35 U.S.C. 112, first paragraph, is respectfully traversed. Specifically, Applicant respectfully disagrees with the Examiner's assertion (page 3, item 6, line 5) that the application allegedly:

> does not provide enablement for all possible "functional groups", for example, "functional groups" having antigen-antibody type specificity.

Claim 1, reproduced in part below, contains several terms which must be interrelated (shown in **bold face**):

> A method for analyzing a complex protein mixture, said method comprising:
> labeling one or more active target proteins present in said complex protein mixture by combining at least one **probe**, said **probe(s)** comprising a **functional group** specific for one or more **active target proteins** and a **fluorescent moiety**, with said complex protein mixture under conditions whereby said **probe(s)** covalently react with said **active target proteins**; ...

Thus, a probe, as defined by claim 1, comprises a functional group and a fluorescent moiety. In addition, the probe is required to covalently react with active target proteins. Accordingly, the functional group component of the probe, which has specificity for one or more active target proteins, must covalently react with the active target protein in order to read on claim 1. In contrast, with respect to the concept of antigen-antibody type specificity (as suggested by the Examiner, page 3, item 6, line 6,) antigen-antibody complex formation does not involve covalent reaction between the two components, instead merely representing interaction between the two components. Thus, covalent reaction encompasses the formation or breaking of covalent bonds, while interaction between antigen and antibody does not.

The rejection of claims 1-6, 8-13, and 37 under 35 USC 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, is respectfully traversed.

Specifically with respect to Item 8a, Applicant respectfully disagrees with the Examiner's assertion (page 3, item 8a) that "[i]n claim 1, it is unclear what is meant by the term 'a functional group specific for one or more active target proteins'." The specificity of the functional group of claim 1 is defined by the requirement that said functional group must be specific for one or more active target proteins, and that the probe comprising the functional group must react with said active target proteins. Accordingly, the functional group undergoes a chemical reaction (Specification, paragraph 67) with the active target protein giving a covalently bound labeling probe. Exemplary amino acids capable of undergoing reaction with exemplary functional group(s) leading to labeling of the active target protein by probe are recited in Specification paragraph 105.

Specifically with respect to Item 8b, Applicant respectfully disagrees with the Examiner's assertion concerning claim 1 that "it is unspecified whether the 'fluorescent moiety' part of the 'probe' or the 'functional group' part of the 'probe' reacts with the 'active target protein.' Additionally, it is unclear what kind of binding is involved in 'reaction',. . ." Contrary to the Examiner's assertion, it is submitted to be clear that the specificity of the functional group results from reaction thereof with specific amino acids of the active target proteins. Thus, the present invention clearly contemplates the type of binding (i.e., covalent binding) between which components (i.e., between a chemically reactive functional group and a targeted amino acid).

Specifically with respect to Item 8c, Applicant respectfully disagrees with the Examiner's assertion concerning claim 1 that "it is unclear what portion of the 'probe' is bound by the 'receptor'." Indeed, the Examiner's concern is respectfully submitted to be misplaced as the exact nature of the binding of "probe" to "receptor" is irrelevant. For example, the Examiner's attention is drawn to Example 6 (Specification, paragraph 177-179) wherein tetramethyl rhodamine (TMR) was covalently attached to keyhole limpet hemocyanin to generate monoclonal antibodies. Those of skill in the art would recognize that TMR functions as a hapten

in the protocol of Example 6, and that antibodies so produced would be assayed for FMR binding affinity.

Specifically with respect to Item 8d, Applicant respectfully disagrees with the Examiner's assertion that "Claim 37 is indefinite in not reciting the specificity of the 'antibody'." Claim 37, which depends on claim 1, is submitted to be clear. Claim 1 requires the receptor to bind the probe which binds the active target protein. Thus, the specificity of the antibody is to the probe. See, for example, Example 6 (Specification, paragraphs 177-179), which specifies a method for the generation of anti-fluorophore antibodies. Moreover, as amended, claim 37 expressly requires antibody which binds probe.

Specifically with respect to Item 8e, Applicant respectfully disagrees with the Examiner's assertion concerning claim 11 that "it is unclear what is meant by the term 'or through a link'." The Examiner's attention is directed to paragraph 41 of Applicant's specification, where the term "linker moiety" is discussed, and refers to a bond or chain of atoms used to link one moiety to another. In the present case of claim 11, said moieties comprise functional group "Y" and functionality "A", each of which are expressly defined in the Specification and claims.

Specifically with respect to Item 8f, Applicant respectfully disagrees with the Examiner's assertion concerning claim 11 that "it is unclear what is meant to be encompassed by the generic terms . . ." Contrary to the Examiner's assertion, each of the generic terms referred to in this paragraph of the Office Action are well known terms of art. Thus, each of the terms "fluorophosphonyl ester", "epoxide", "ethylene alpha to an activating group" and "halogen beta to an activating group" are submitted to be readily understood by those of skill in the art. Exemplary compounds are found in the Specification at e.g., paragraph 79.

Specifically with respect to Item 8g, the Examiner's observation of potentially ambiguous language in claim 12 is noted. Thus, in view of the amendment to claim 11 submitted herewith,

renaming the variable "F" to the variable "Y", it is submitted to be clear that the "variable" of claim 12 is not a variable at all -- "F" is the chemical symbol for the element fluorine.

Specifically with respect to Item 8h, Applicant respectfully disagrees with the Examiner's assertion concerning claim 1 that "it is unclear what is meant by the term 'analyzing a complex protein mixture'." The Examiner's attention is directed, for example, to claim 2, which explicitly sets forth the following correlation: "said fluorescent signal indicates the presence of an active target protein in said complex protein mixture." Thus, it is respectfully submitted to be clear that "analyze" means to detect an analyte, in this case the presence of an active target protein.

Specifically with respect to Item 8i, claim 11 has been amended herein to delete the word "and" from the phrase "and/or"--thus bringing the text into harmony with the structure which shows attachment of Fl at the 2' or 3' positions, but not both.

Specifically with respect to Item 8j, Applicant respectfully disagrees with the Examiner's assertion that claim 12 is allegedly indefinite and incomplete in not defining the variable "F". As noted above, the "variable" is not a variable at all -- "F" is the chemical symbol for the element fluorine.

The rejection of claims 1-6, 8-10, 13 and 37 under 35 USC § 102(b) as allegedly being anticipated by, or, in the alternative, as allegedly being obvious over Minden et al (US 6,127,134) is respectfully traversed. Specifically, the present invention, as defined for example by claim 1, distinguishes over Minden by requiring a method for analyzing a complex protein mixture, said method comprising the steps of:

labeling one or more active target proteins present in said complex protein mixture by combining at least one defined probe,

isolating one or more of said labeled active target proteins by binding to a

receptor bound to a solid phase;

removing unbound proteins;

releasing bound labeled active target proteins from said receptor; and

detecting a signal from one or more labeled active target proteins present in said complex protein mixture following said isolating.

Minden does not disclose such a method. For example, with respect to the isolation of one or more labeled active target proteins by binding to a receptor bound to a solid phase, Minden is silent. Moreover, Minden does not use probes specific for "active target proteins." Instead, Minden uses probes that will react with a particular amino acid side chain (e.g., lysine or cysteine) regardless of the functional state of the protein. For example, the NHS reactive group will react with all lysine residues rapidly under the conditions described by Minden. Thus, Minden does not anticipate the present claims.

Neither does Minden render the present claims obvious. No motivation has been provided to modify the teaching of Minden so as to arrive at the present invention. Accordingly, the present claims are not obvious over Minden.

The rejection of claim 12 under 35 USC § 102(b) as allegedly being anticipated by Bronstein (US 4,978,614) is respectfully traversed. Specifically, the present invention, as defined by claims 1 and 12, distinguishes over Bronstein by requiring covalent attachment of label such that labeled active target protein(s) can be subsequently separated by electrophoresis. Bronstein neither discloses nor suggests such attachment. Because of this difference, if the Bronstein method were applied to a complex protein mixture, there would be no way to determine which protein(s) in the mixture had performed a reaction on the dioxetane reporter.

Whereas the labeling in the present invention is covalent, the Bronstein labeling is not covalent. Whereas the labeled compounds of the present invention can be separated electrophoretically, the Bronstein compounds cannot be separate electrophoretically due to the

lack of covalent attachment. Whereas claim 12 requires phosphinyl- and phosphonyl-fluorides (i.e., but not phosphate esters as alleged by the Examiner, where such esters require three oxygens bound to phosphorous), Bronstein requires the dioxetane nucleus. Accordingly, Bronstein does not anticipate the present invention.

In view of the amendments and remarks provided herein, it is respectfully submitted that the present application is now in condition for allowance. Accordingly, reconsideration and favorable action on all claims is respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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FOLEY & LARDNER LLP
Customer Number: 30542
Telephone: (858) 847-6714
Facsimile: (858) 792-6773

Stephen E. Reiter
Attorney for Applicant
Registration No. 31,192